High-throughput image-based shoot phenotyping at OloPhen

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CZPPN

CZECH PLANT PHENOTYPING NETWORK

C. R. HANÁ

- High-throughput bioassaying in cotrolled conditions
- Development of protocols and specialized software for image analysis and data processing
- Field trials and field phenotyping

Photon Systems Instruments

- Development of scientific instruments for research in biological and agricultural sciences
- Development and manufacturing innovative technology for cultivation of plants and for non-invasive complex analysis of various plant traits in fully automated manner

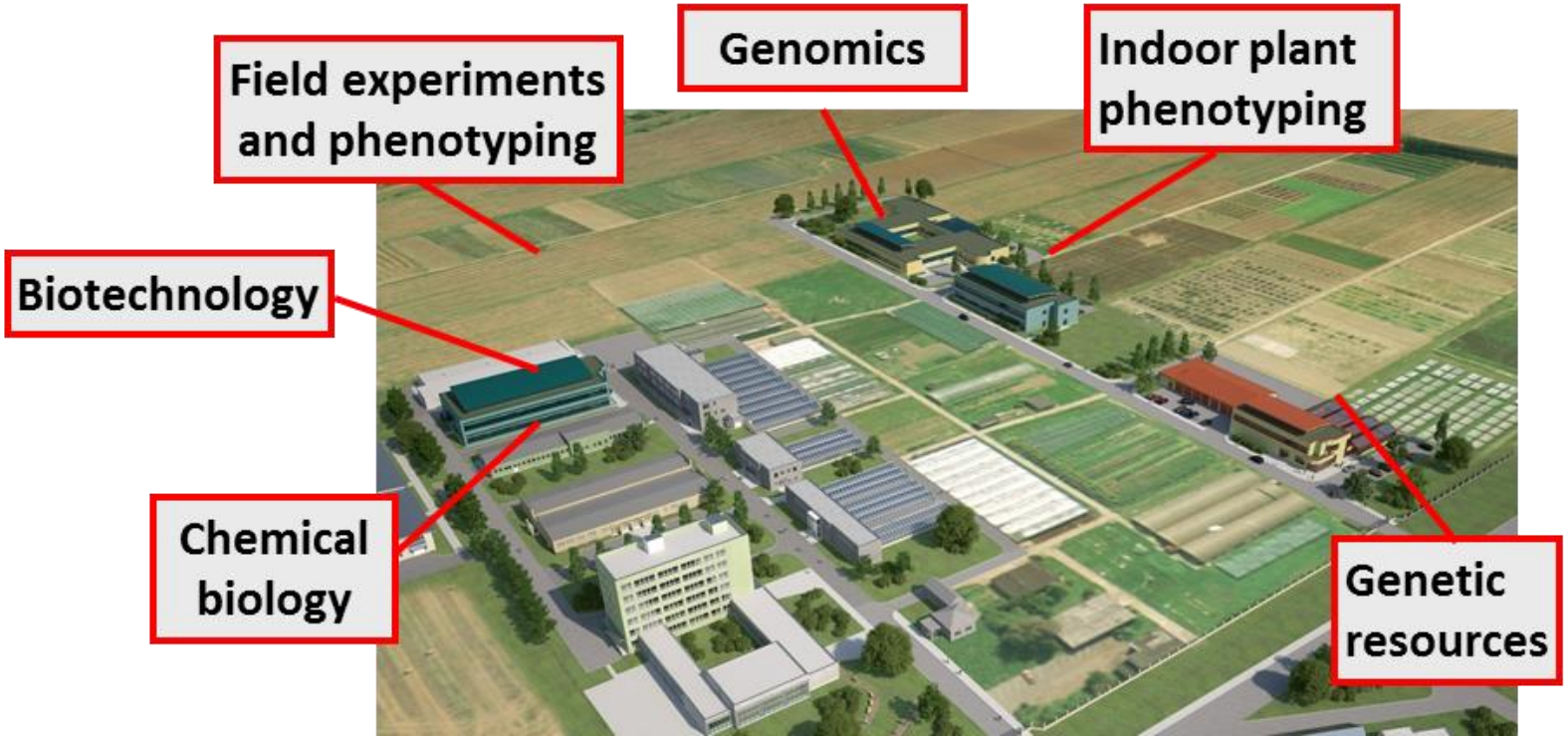
CEITEC

Central European Institute of Technology

BRNO | CZECH REPUBLIC

- Generating basic knowledge on the molecular mechanisms allowing integration of various signaling pathways
- Development and implementation of technologies to improve plant stress tolerance
- Phenotyping on cellular and tissue level

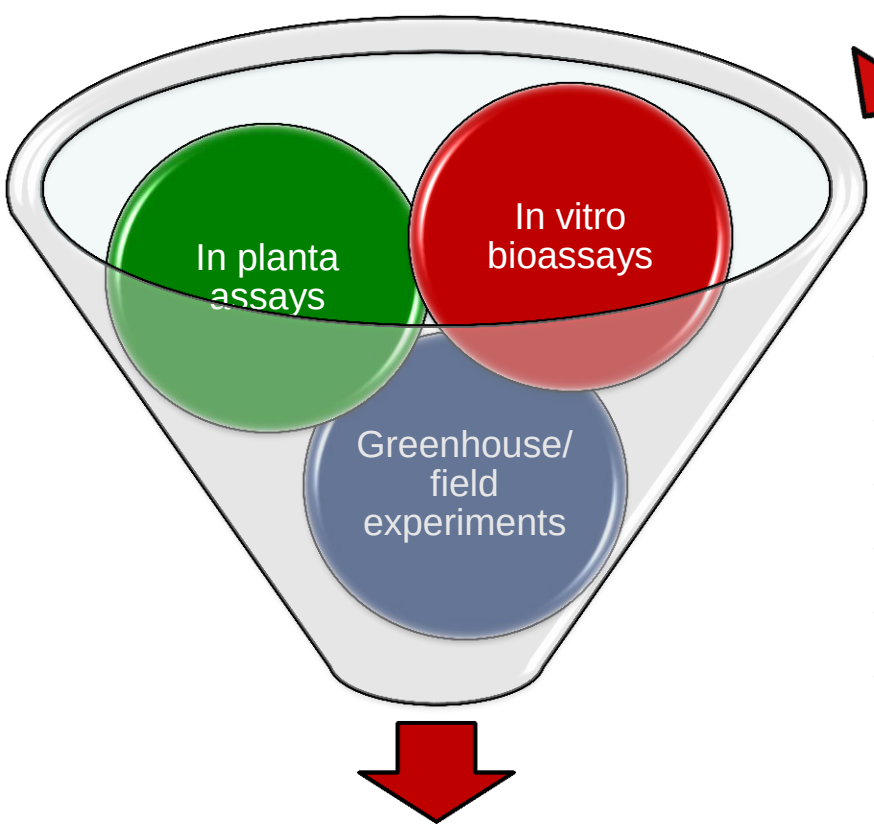
Centre of the Region Haná for Biotechnological and Agricultural Research, UPOL



- Development and testing of new plant growth regulators
- Development and implementation of protocols for HTS and large-scale bioassaying and indoor phenotyping
- Field trials and field phenotyping

OloPhen

Automated bioassaying and phenotyping pipe-line



- In vitro bioassays
- In planta assays
- Integrative phenotyping
- Greenhouse/field experiments
- Transcriptomics
- Metabolomic profiling

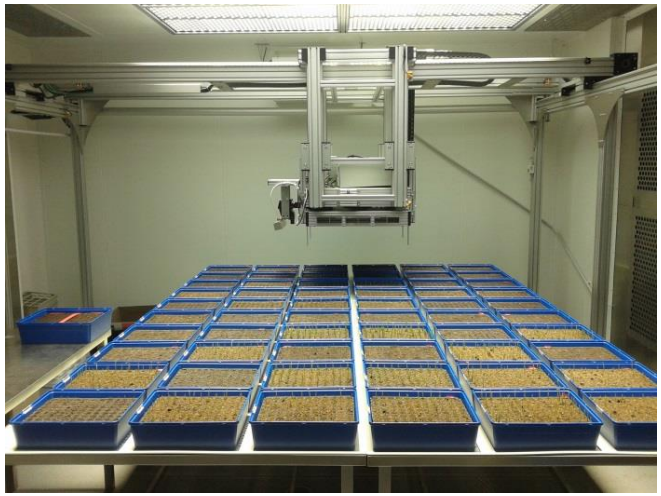
Identification of new plant growth regulators and their mode of action

- High-throughput
- Automation
- Non-invasive methods

OloPhen

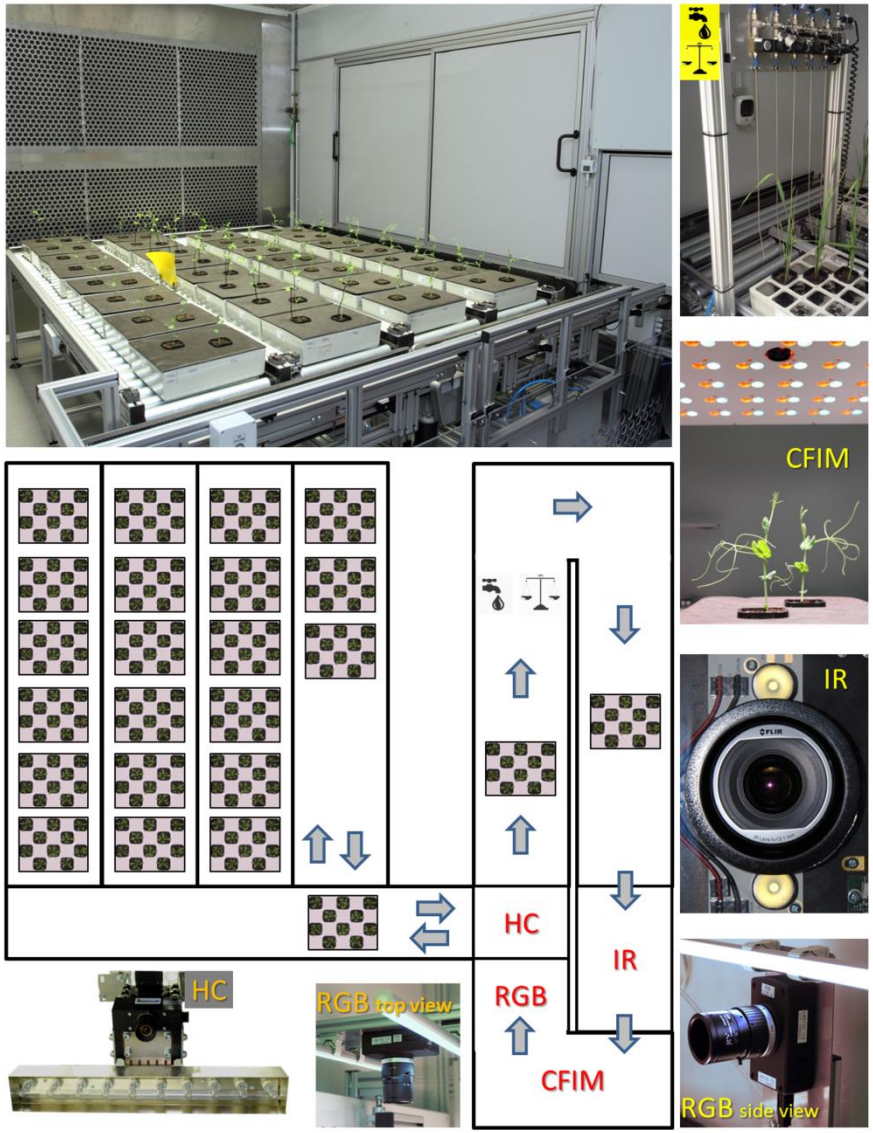
Top view imaging system XYZ PlantScreen™

- Controlled conditions
- plant growth sensor (RGB top view high-resolution camera with homogenous LED lightning)
- sensors of physiological responses:
  - FluorCam unit – Chl flurescence kinetic analysis
  - hyperspectral unit (VIS 380-1000 nm)
- capacity: 7.5 square metres (480 culture multiwell plates, 60 trays, 1200 standardized Arabidopsis pots)



OloPhen
Conveyor PlantScreen™ system

- Controlled conditions
- three RGB cameras, FluorCam, thermoimaging, hyperspectral imaging (1000-2500 nm), acclimation cabinet, automatized pot weighing and watering
- capacity: 640 plants for top-view experiments, 64-32 plants for three-views experiments



Humplík JF, Lazar D, Husičková A, Spíchal L (2015) Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses – a review. *Plant Methods,* 11:29.

Automated bioassaying and phenotyping pipe-line

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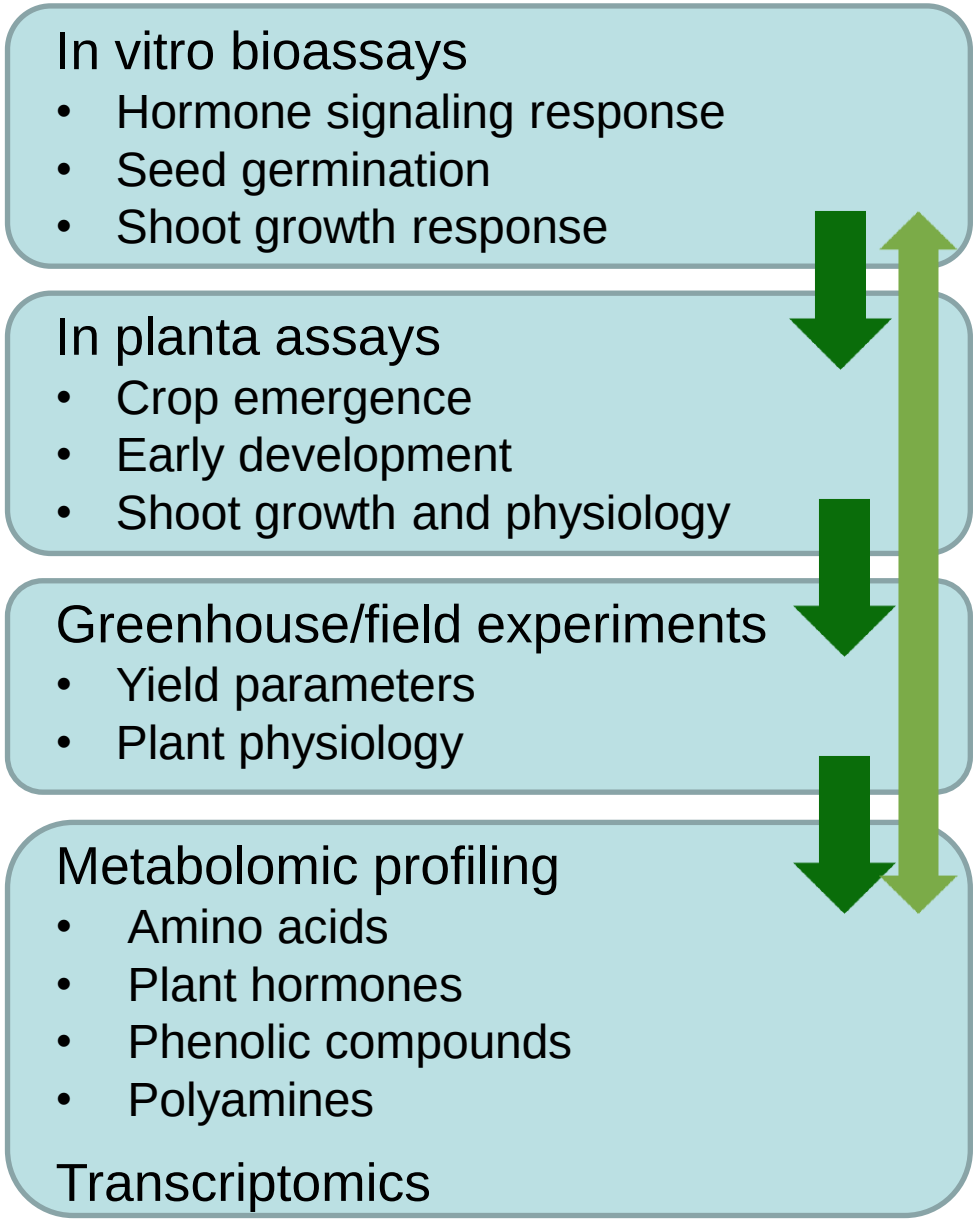
We test

- genotypes
- Compound libraries
- Extracts
- Compounds/mixtures
- PGPR
- microbes
- VCs
- …
- Commercial products
  - Series
  - Libraries
  - Batches
  - …
- Way of application
  - Seed treatment
  - drench
  - foliar



Automated bioassaying and phenotyping pipe-line

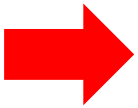
In vitro bioassays

In planta assays

Greenhouse/field experiments

Metabolomic profiling

Transcriptomics



Identification of new plant growth regulators/biostimulants and their mode of action

- In normal conditions
- In stress conditions
  - Salt
  - Drought
  - Temperature
  - Light intensity
  - Nutrients

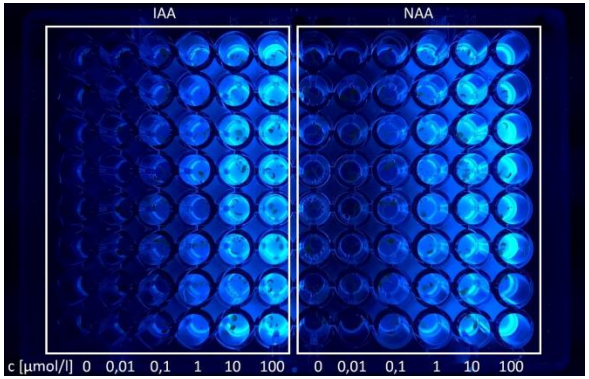
In vitro bioassays

- Hormone signaling response
- Seed germination
- Shoot growth response

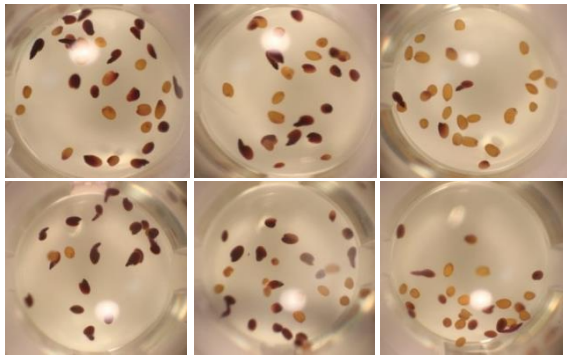
Characteristics:
- Fast (in days)
- High-throughput (hundreds of variants)
  - Time series design (kinetic)
  - High number of combinations
  - High number of replicates
  - Population behavior
  - Statistical approaches

Using multiwell plates
- 6-, 24-, 48-, 96-well plates
- Non-invasive simple readout

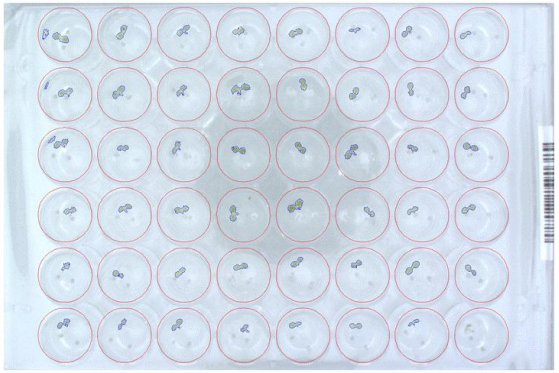
Hormone signaling response



Seed germination



Shoot growth response



In vitro bioassays – Shoot growth response

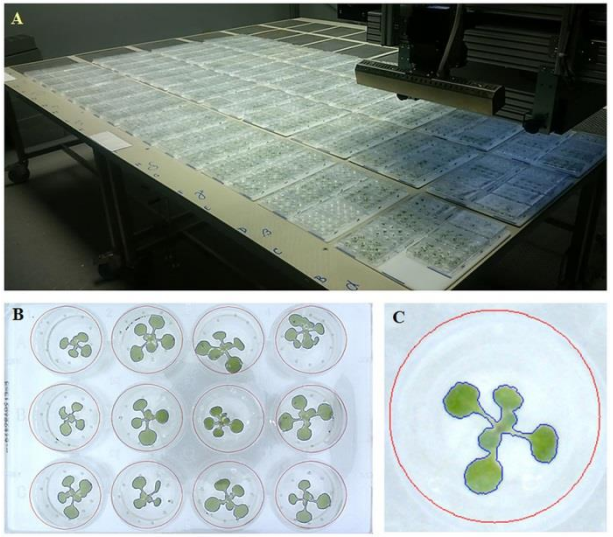
- Analyses of effect on shoot area of Arabidopsis
  - Stimulation/Inhibition of shoot growth
  - normal conditions / Interaction with stress conditions
    - Salt, temperature, nutrition, drought

METHODS ARTICLE
Front. Plant Sci., 04 October 2017 | https://doi.org/10.3389/fpls.2017.01702

An Automated Method for High-Throughput Screening of *Arabidopsis* Rosette Growth in Multi-Well Plates and Its Validation in Stress Conditions

Nuria De Diego[1], Tomáš Fürst[1], Jan F. Humplík[1,2], Lydia Ugena[1], Kateřina Podlešáková[1] and Lukáš Spíchal[1*]

[1]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Olomouc, Czechia
[2]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Czech Academy of Sciences, Olomouc, Czechia



„This approach will allow simultaneous testing of a large number of potentially bioactive compounds in a wide range of concentrations and/or genotypes, under various growth conditions.“ (De Diego et al., 2017)

Top view imaging system XYZ PlantScreen™

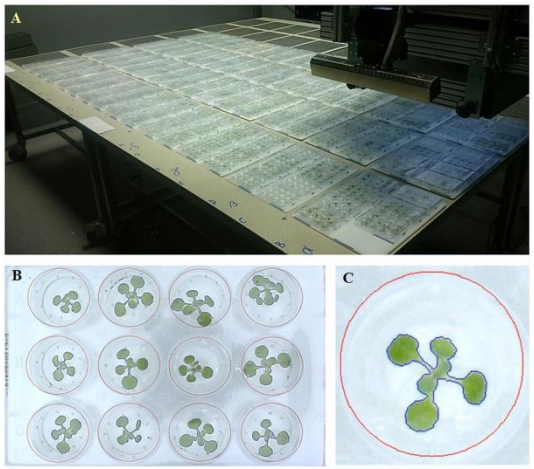
frontiers in Plant Science

ORIGINAL RESEARCH
published: 13 September 2018
doi: 10.3389/fpls.2018.01327

Characterization of Biostimulant Mode of Action Using Novel Multi-Trait High-Throughput Screening of *Arabidopsis* Germination and Rosette Growth

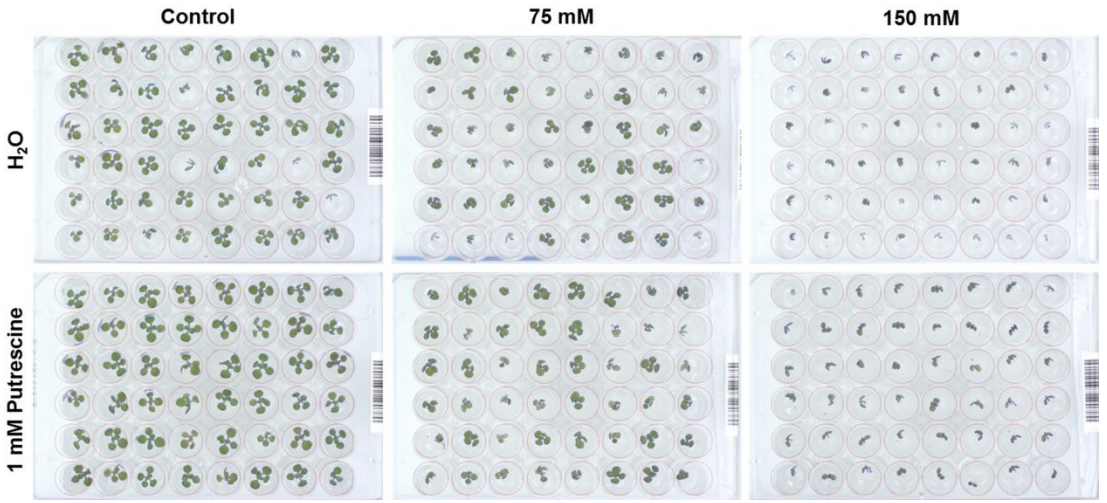
Lydia Ugena[1†], Adéla Hýlová[1†], Kateřina Podlešáková[1], Jan F. Humplík[1,2], Karel Doležal[1], Nuria De Diego[1] and Lukáš Spíchal[1]*

6-, 12-, 24-well plates



De Diego et al., 2017

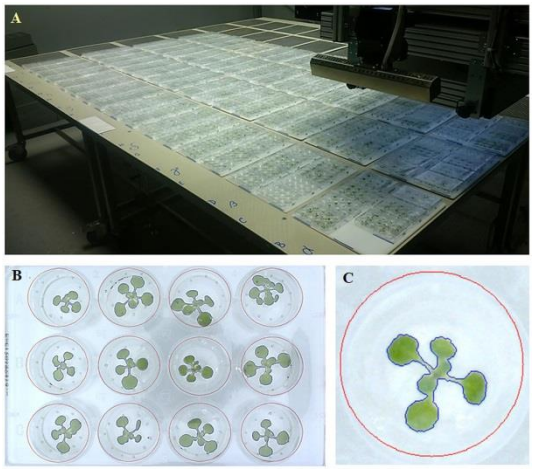
48-well plates



Ugena et al., 2018

Top view imaging system XYZ PlantScreen™

6-, 12-, 24-well plates



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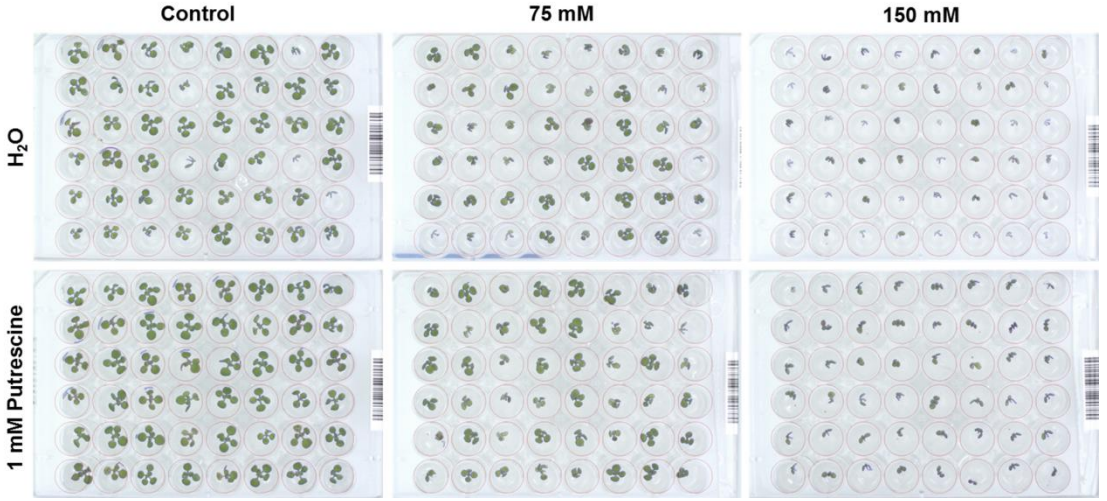
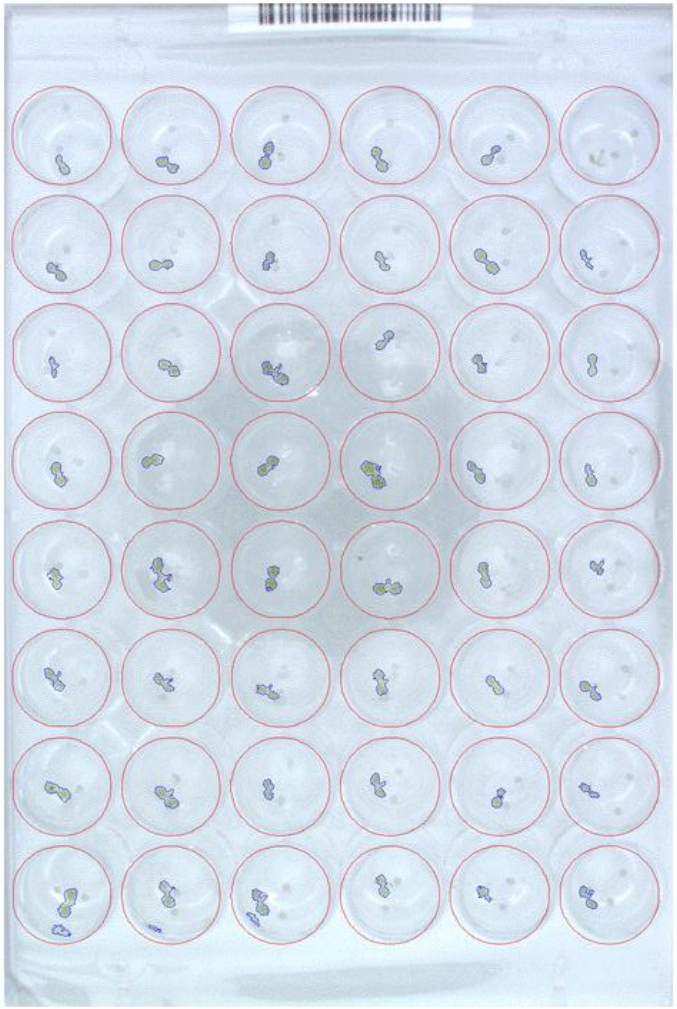


Ugena et al., 2018

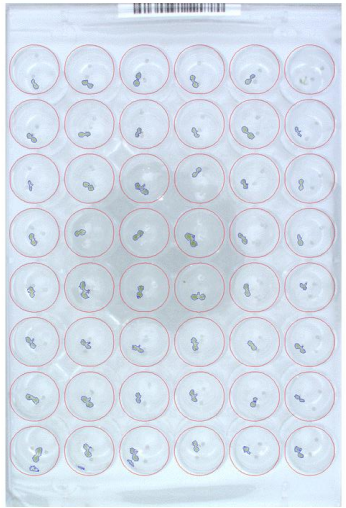
Type of well plate	No. plants	Replicates	Platform capacity	Total plants	No. variants	Assay duration
6-Well Plates	6	3		2880	160	14 days
12-Well Plates	12	2	480 Plates	5760	240	9 days
24-Well plates	24	1		11520	480	9 days

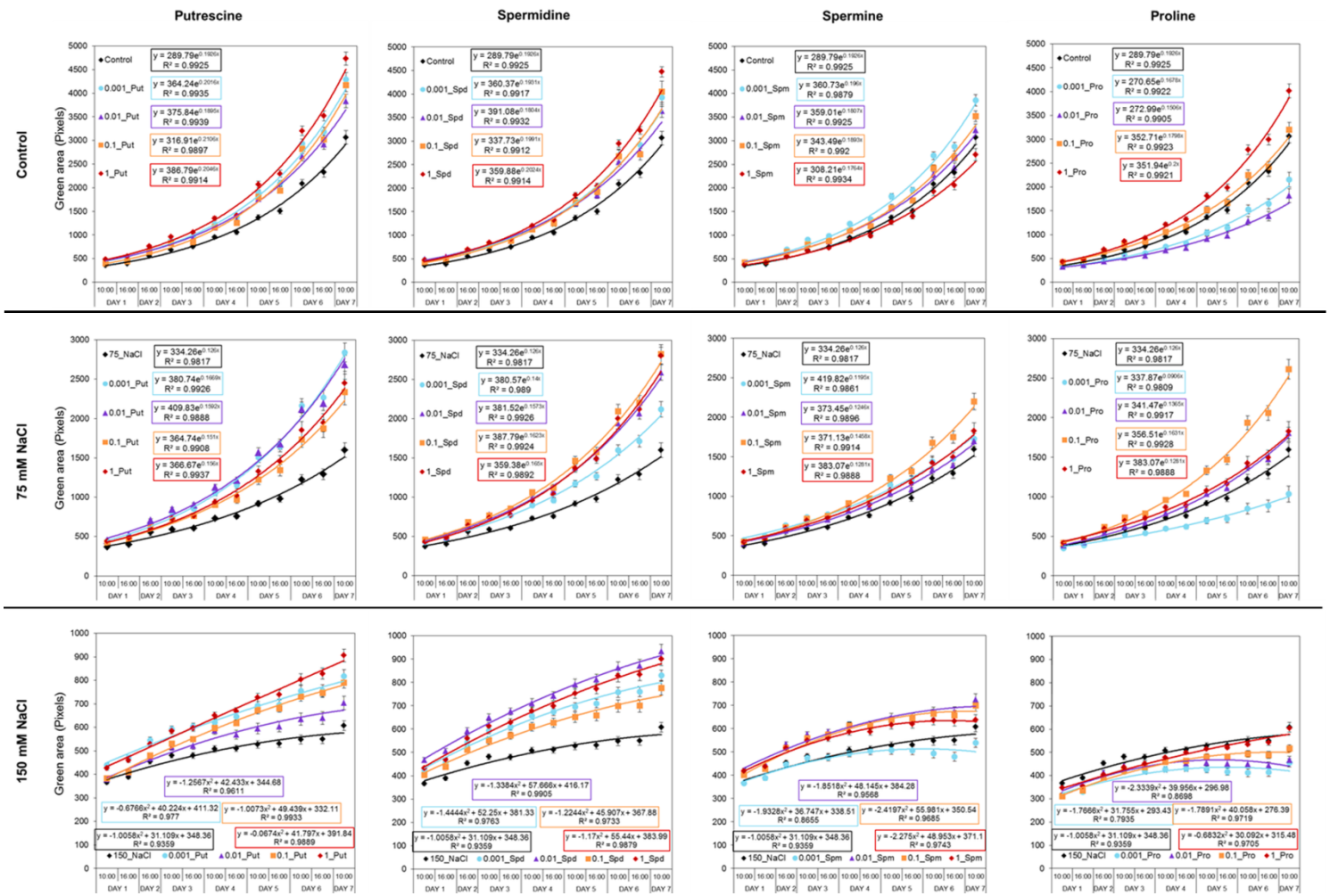
48-well plates	23040 plants	7 days

In vitro bioassays – Shoot growth response



In vitro bioassays – Shoot growth response





4 cpds x 4 concentrations x 3 conditions = 48 variants (2.304 plants)

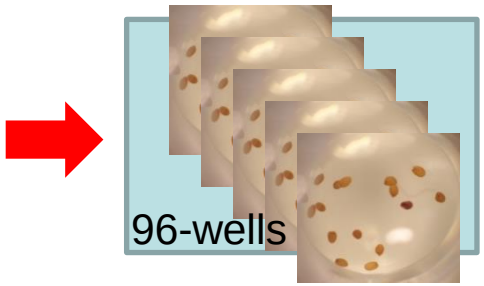
L. Ugena, A. Hýlová, J. F. Humplík, K. Podlešáková, K. Doležal, N. De Diego, L. Spíchal, 2018

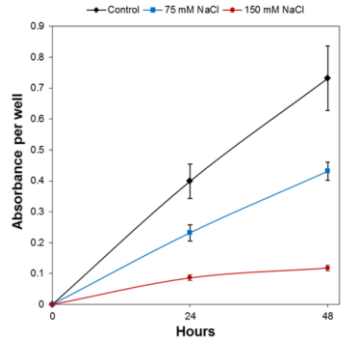
In vitro bioassays – Seed germination

- Analyses of effect on seed germination of Arabidopsis
  - Stimulation
  - Inhibition
  - In normal conditions
  - Interaction with stress conditions
    - Salt, temperature, heavy metals

- Time/concentration-dependent response is analyzed
- In normal/stress conditions

- Using 96-well plates and robotic pipetting station
- Seed counting and non-invasive colorimetric readout (MTT reduction)
- Time/concentration-dependent response curve (80 seeds per variant)





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Multi-**t**rait **h**igh-**t**hroughput **s**creening of *Arabidopsis* germination and rosette growth

MTHTS

Traits:
Germination rate
Early seedling establishment
Growth capacity
Leaf colour index

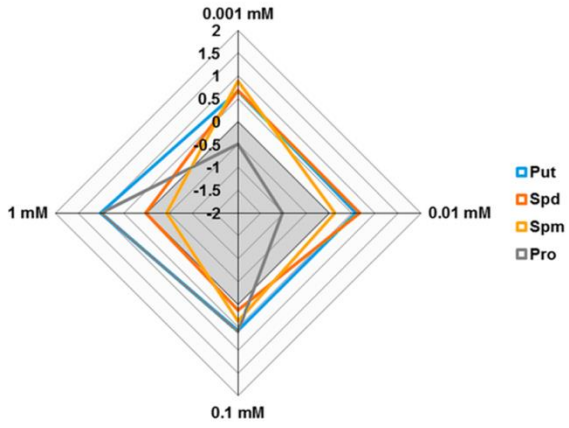
Plant Biostimulant Characterization Index (PBC)

PBC index- Control				
Concentration	Put	Spd	Spm	Pro
0.001 mM	0.67	0.68	0.89	-0.49
0.01 mM	0.58	0.66	0.12	-1.03
0.1 mM	0.57	0.12	0.37	0.59
1 mM	1.01	0.02	-0.44	0.99

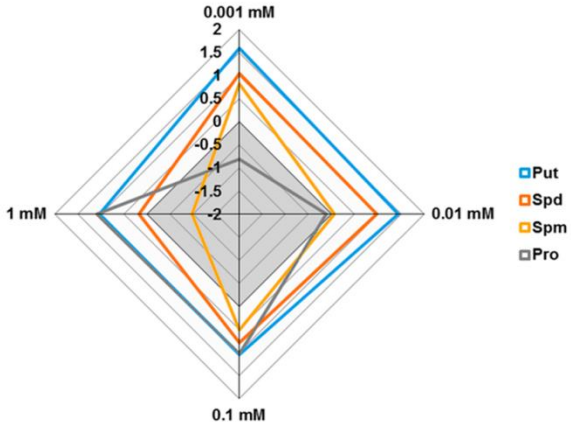
PBC index- 75 mM NaCl				
Concentration	Put	Spd	Spm	Pro
0.001 mM	1.59	1.04	0.82	-0.81
0.01 mM	1.44	0.98	0.06	-0.12
0.1 mM	1.04	0.78	0.51	1.03
1 mM	1.03	0.17	-0.98	1.08

PBC index- 150 mM NaCl				
Concentration	Put	Spd	Spm	Pro
0.001 mM	1.27	1.24	0.27	-0.55
0.01 mM	1.28	1.17	1.00	-0.31
0.1 mM	1.44	1.05	0.86	0.62
1 mM	1.70	0.89	-0.09	0.90

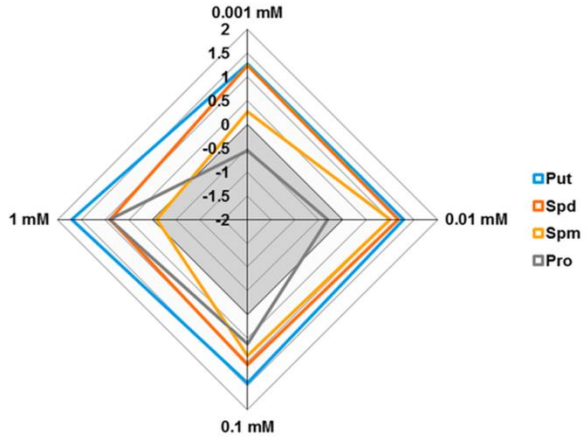
A



B



C



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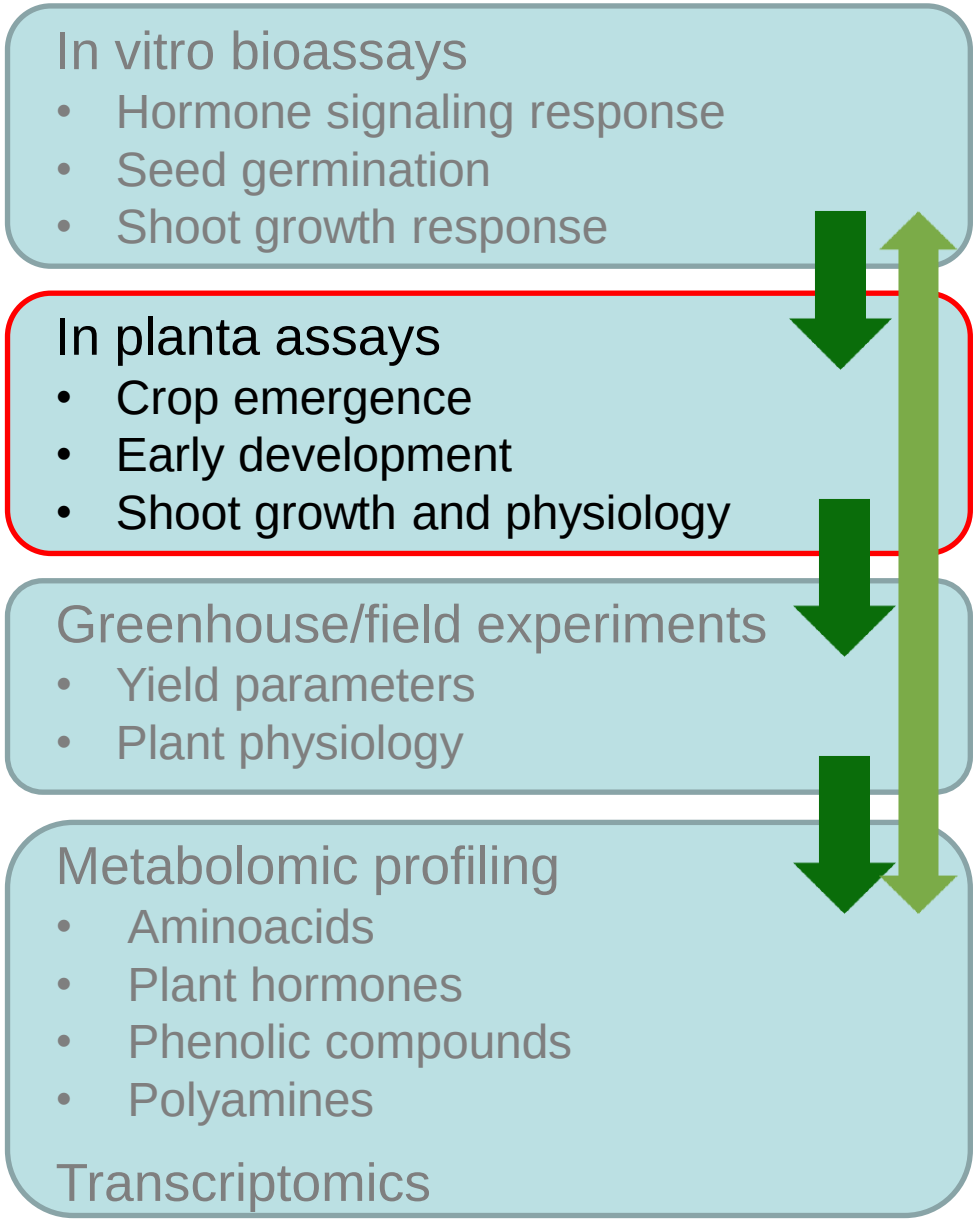
Automated bioassaying and phenotyping pipe-line

C. R. HANÁ

We test

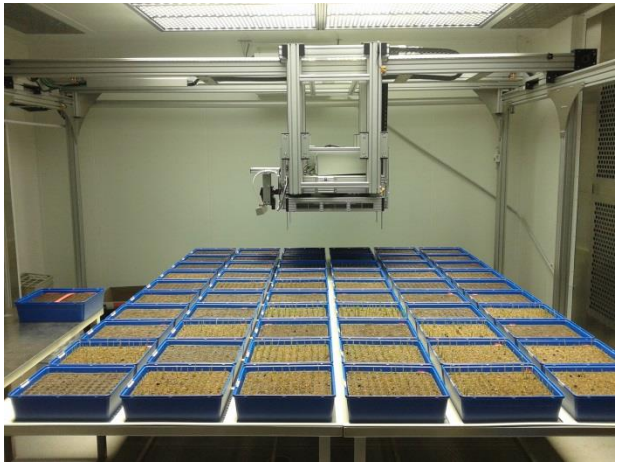
- genotypes
- Compound libraries
- Extracts
- Compounds/mixtures
- PGPR
- microbes
- VCs
- …
- Commercial products
  - Series
  - Libraries
  - Batches
  - …
- Way of application
  - Seed treatment
  - drench
  - foliar



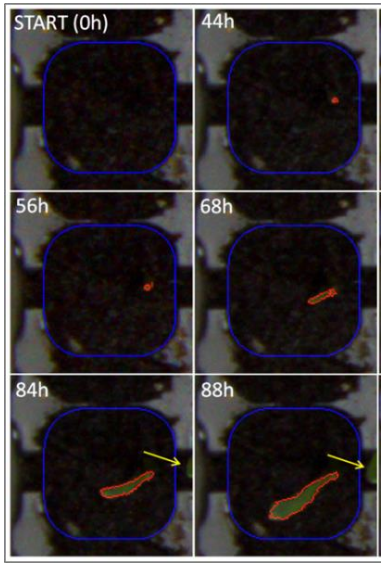
In planta assays - crop emergence

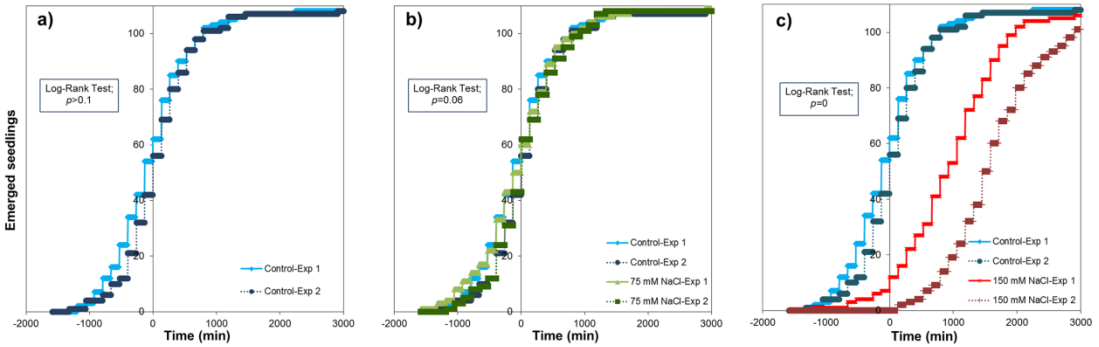
- Testing of the emergence of crops (cereals, tomato, rapeseed) after application of various compounds and/or stress conditions (salt, cold, drought)
- Seed treatment
- 60 variants X 110 seeds
- Screening each 2 hours for approx. 7 days

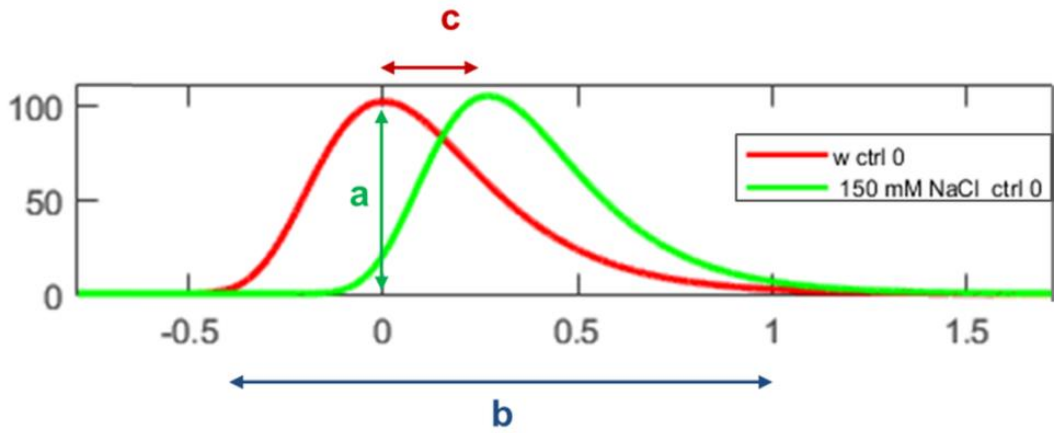
Time/concentration-dependent response of a biostimulant effect in normal/stress conditions during the heterotrophic growth



CroSeEm: An automated approach of high-throughput dynamic scoring of crop seedling emergence







Three traits analyzed:

a) Total seedlings emerged

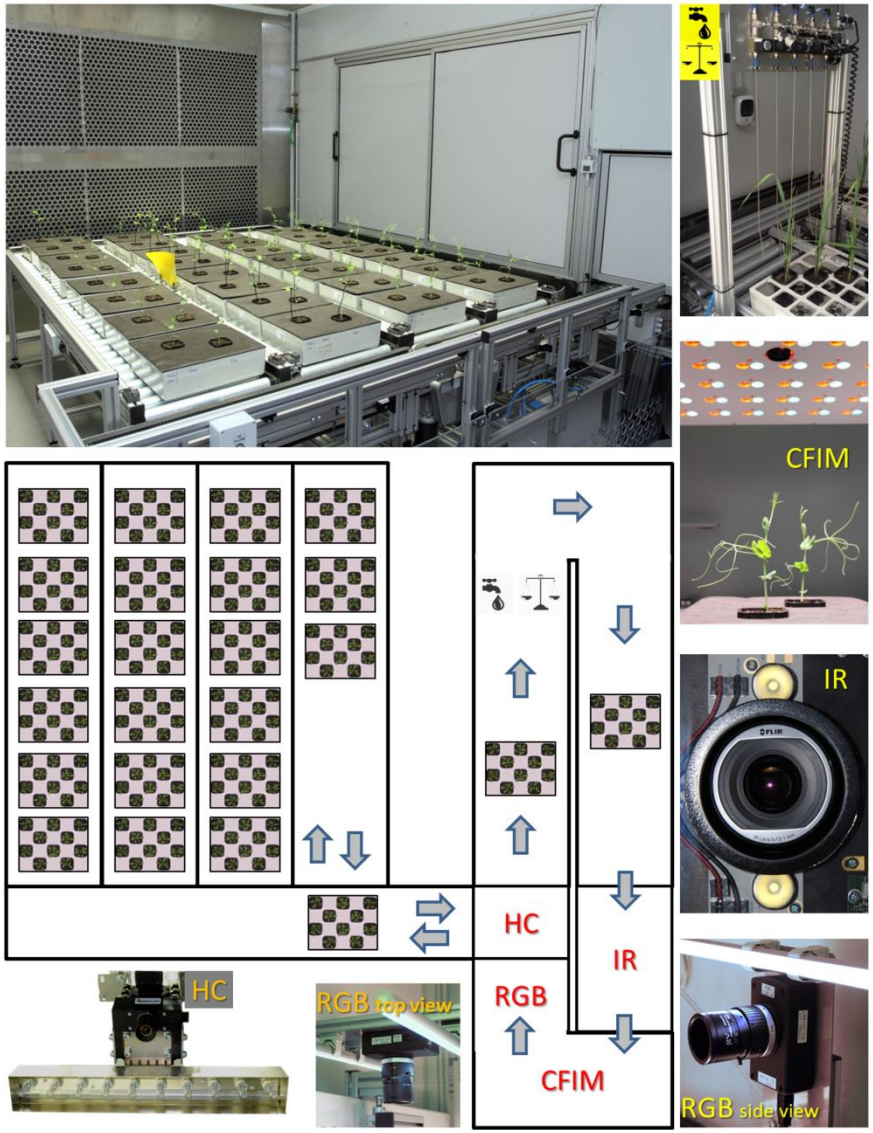
b) Speed (homogeneity of population)

c) Time lag

L. Ugena, J. F. Humplík, T. Fürst, N. De Diego, L. Spíchal, under review

Conveyor PlantScreen™ system – integrative phenotyping

- three RGB cameras, FluorCam, thermoimaging, hyperspectral imaging (1000-2500 nm), acclimation cabinet, automatized pot weighing and watering
- capacity: 640 plants for top-view experiments, 64-32 plants for three-views experiments

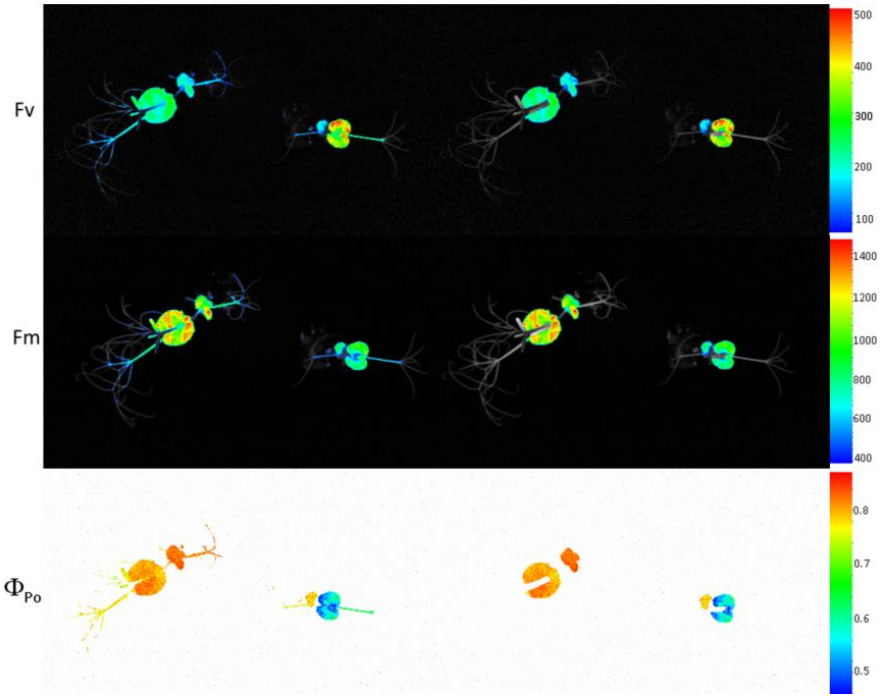


Humplík JF, Lazar D, Husičková A, Spíchal L (2015) Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses – a review. *Plant Methods,* 11:29.

In planta assays – early development

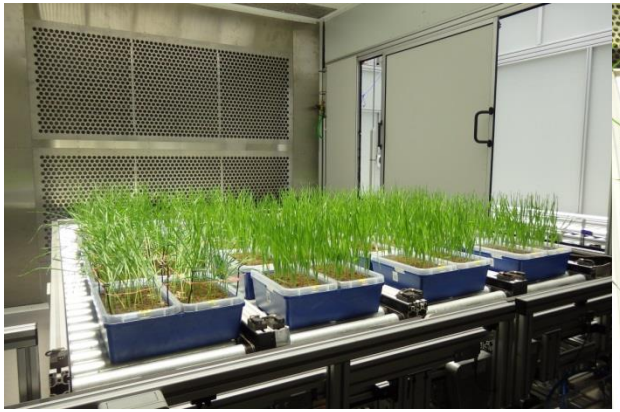
- Crop stress response
- Growth and physiological traits, and strategies of cold tolerant varieties of pea

Humplík JF, Lazár D, Fürst T, Husičková A, Hýbl M, Spíchal L (2015) Automated integrative high-throughput phenotyping of plant shoots: a case study of the cold-tolerance of pea (Pisum sativum L.). *Plant Methods,* 11:20.

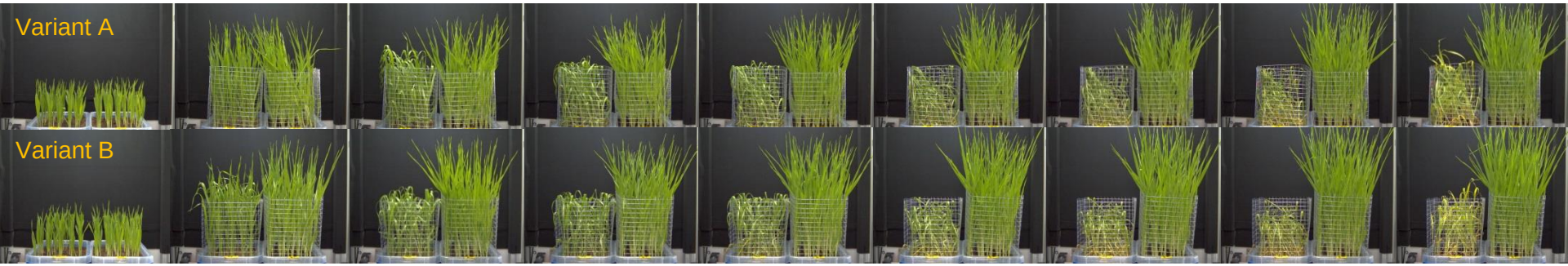


In planta assays – early crop development

- Crop stress response
- Drought, salinity, cold
- Fast assay using RGB imaging



Drought tolerance assay with crops



15 days

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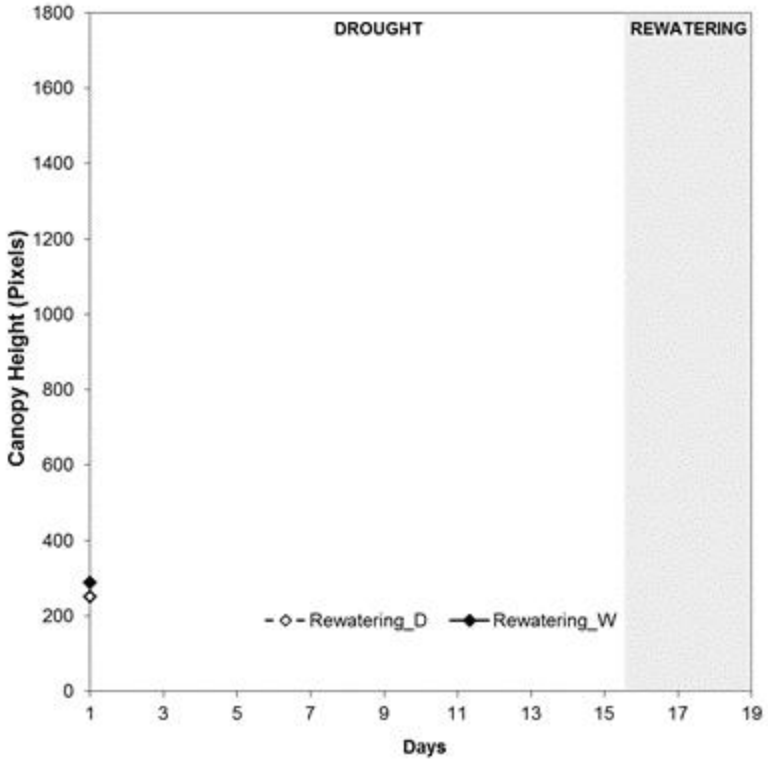
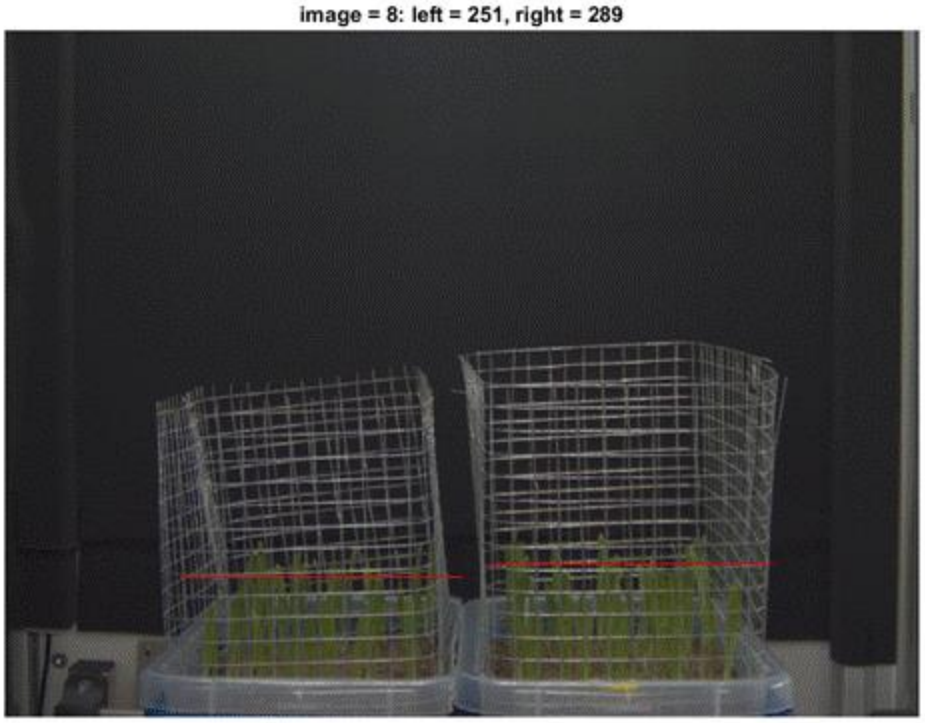
Crop drought tolerance assay





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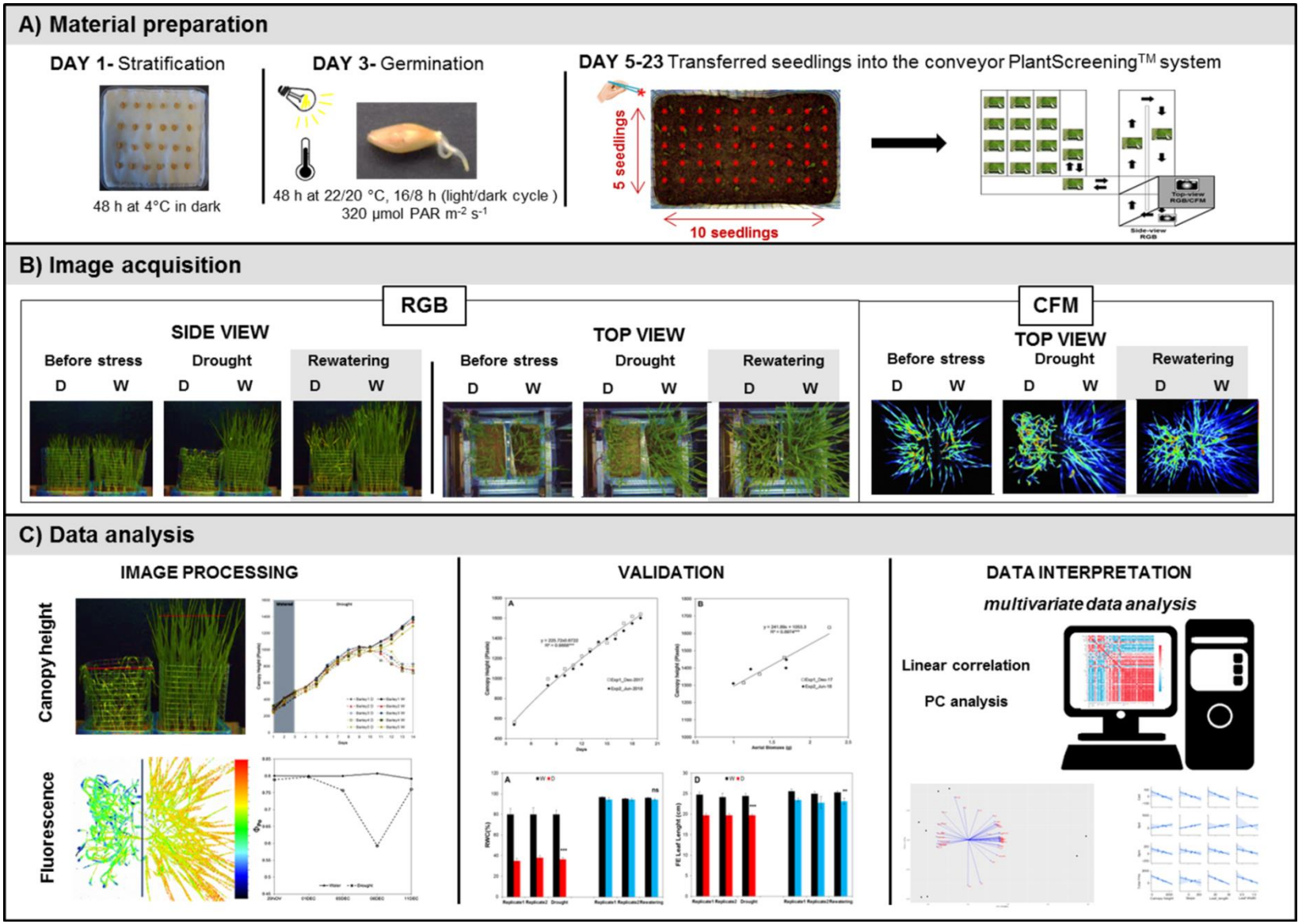

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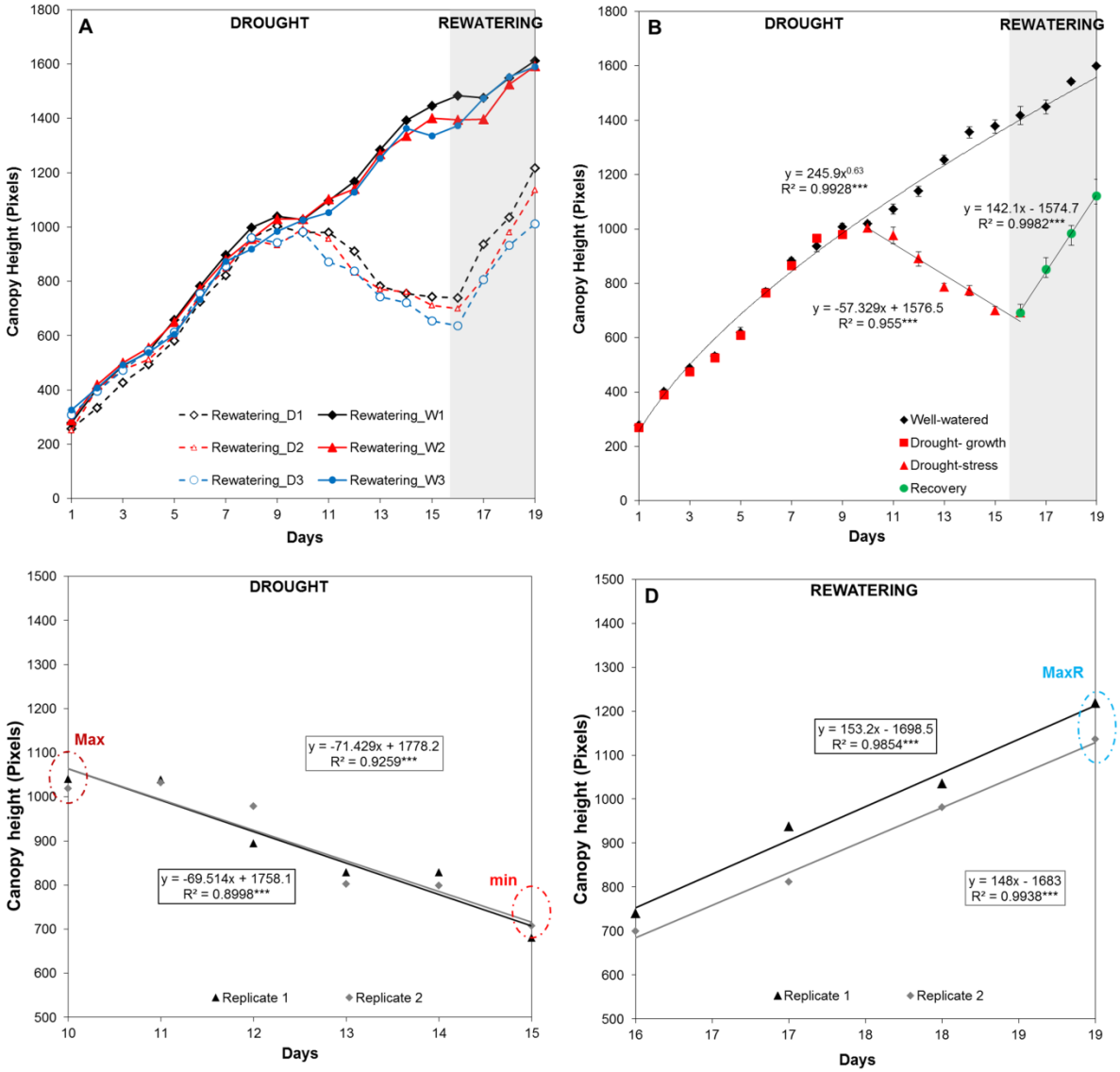

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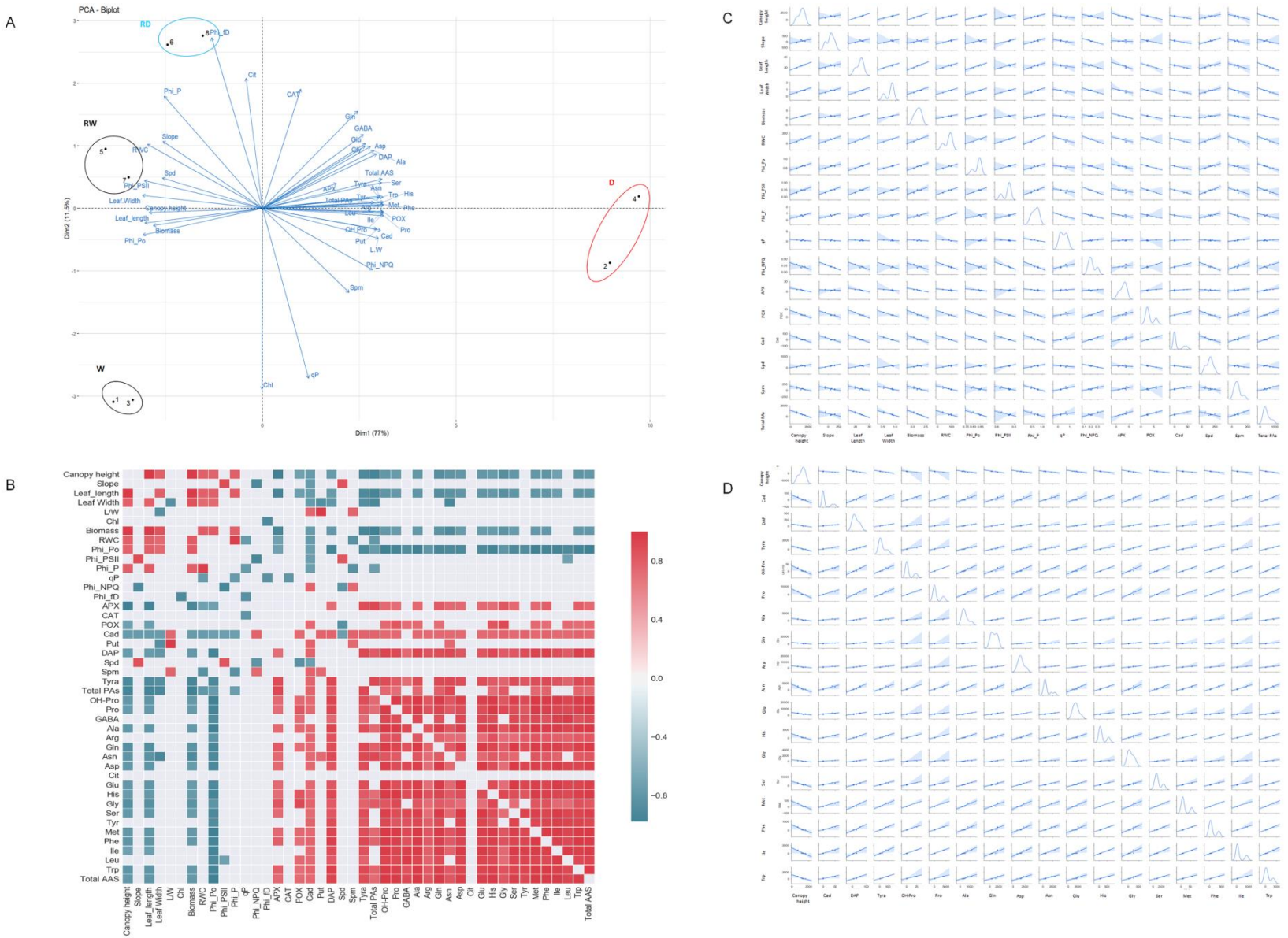

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Thanks to

- Nuria De Diego
- Jan F. Humplík
- Tomáš Fürst
- Lydia Ugena
- Cintia Marchetti
- Katka Podlešáková
- Adéla Hýlová
- Petr Kuczman
- Alexandra Husičková
- Dušan Lazár
- Jana Vašková